ANTIBIOTICS—20 YEARS LATER*

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THE causes of the majority of diseases that afflict man, his herds, and his crops, were still unknown as late as a century ago. There were at that time only very few, among both the scientists and the medical practitioners, who surmised the contagious nature of infectious diseases. It is true that numerous theories were afloat, postulating the existence of living contagious agents, but exact proof was lacking. About 1860 the great physiologist, Claude Bernard, wrote that "the present state of medical practice suggests that a solution [to the problem of conserving health and of curing disease] is still far to seek." Little did Bernard dream that both the understanding of the causes of most infectious diseases and methods for combatting them were soon to follow. His own protégé, Louis Pasteur, was to become the leader in the new concept of microbes, both as causative agents and as possible means for treating infections. Prophylaxis by means of vaccination and serotherapy was soon to be followed by true chemotherapy. It is the latter, or rather one phase of it, with which I am concerned here.

Just a little over 40 years ago, or towards the end of and soon after World War I, I was personally concerned, on the one hand, with the problem of manufacturing Salvarsan, the first true chemotherapeutic agent that found extensive application in the treatment of one of the most serious infectious diseases, and, on the other, with the study of the microbiological population of the soil, particularly involving a little-known group of microorganisms, the actinomycetes. I thus came to visualize, early in my scientific career, the great potentialities of chemotherapy in the treatment of infectious diseases. As I watched the unfolding of the great biochemical potentialities of the actinomycetes, I little dreamed that the time would come when the two so apparently distinct phases of my career would in time be joined together to yield some of the greatest tools for combatting infectious diseases placed at the disposal of man, the antibiotics.

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Twenty years have passed following these first observations. The world was now approaching another World War. In the meantime, the sulfonamides had been discovered. Here were new and better drugs. But still they were not, at that time at least, the most ideal agents for combatting numerous bacterial and other infections, many of which were not even sensitive to them. The study of the biochemical activities of the actinomycetes had made only little progress during that period. It is true that definite evidence was being submitted to the fact that these organisms possess powerful mechanisms for inhibiting the growth of various bacteria and fungi. The possible utilization of such mechanisms in the control of infections was gradually becoming recognized.

The years 1939-1940 were momentous ones in the history of antibiotics. Three important discoveries brought to a head the gradually accumulating information concerning the great potentialities of several groups of microorganisms to produce chemical substances that have the capacity to inhibit the growth of different disease-producing bacteria and fungi. These substances were shown to be active not only in the test tube, but also in experimental animals. A group of aerobic spore-forming bacteria was found by Dubos capable of producing antimicrobial substances, designated as tyrothricin. The capacities of certain fungi to form antimicrobial agents was reemphasized through the rediscovery of penicillin by Florey and Chain. The abilities of actinomycetes as producers of powerful antibacterial and antifungal agents resulted in the isolation of actinomycin in crystalline form in our own laboratories. Of these three types of compounds, penicillin soon began to make history. Tyrothricin remained what came to be known as a "minor" antibiotic. Actinomycin was found to be too toxic for therapeutic use; however, the ease of its formation and isolation encouraged us to search for other antimicrobial substances produced by the same group of organisms, namely the actinomycetes, leading finally to the isolation of streptomycin in 1943.

The following five years witnessed the laying of the groundwork for this new branch of science, resulting in tremendous developments in medical and veterinary science, in animal nutrition, and in the preservation of food and other biological materials. The very name "antibiotic" was introduced at that time. Penicillin was now manufactured on a large scale; greater yields and new forms were being obtained. The actinomycetes were yielding one new antibiotic after

another. Whereas tyrothricin and penicillin were active largely upon diseases caused by cocci and gram-positive bacteria, streptomycin was also active upon diseases caused by various gram-negative bacteria causing tularemia, undulant fever, typhoid, dysentery, plague, cholera, and a variety of other infections. Most important of all, however, was its ability to act upon the tuberculosis organism.

It is now exactly 15 years since the effectiveness of streptomycin in the treatment of tuberculosis was established. This powerful drug proved to be highly effective against the ancient enemy of man, the Great White Plague, to which a death blow was thus delivered. Although streptomycin had its limitations, it pointed the way, thus leading later to the isolation of other antibiotics and synthetic compounds that would tend to overcome them. Who could ever dream that less than a decade and a half later, the following statement would be made:

> "The balance between the tubercle bacillus and man has finally given way in favor of man. The decline in the number of infections and in the number of active cases and deaths is a very real indication of this change in balance. The continuity of this trend and its extent, increasing as it has in recent years, justify the conclusion that the balance has already been so far disturbed as to give the highest hope for complete control of the disease. . . . We cannot say exactly when control will be complete, but there is every indication that it will be some time in the course of the next 20 years" (L. Dublin).

The same idea was further emphasized in a recent issue of the WHO *Chronicle* (November, 1959):

> "The new situation created by the advent of potent antituberculous drugs —first streptomycin in 1944, then PAS and isoniazid—has come upon the world almost unawares, and the profound implications it carries are only just beginning to be grasped. For millions of people throughout the world there is now hope of recovery, whereas previously there was none. The medical profession too is now faced with a change in methods of treatment and prevention of tuberculosis that may alter its approach to problems of control."

But to continue my story. Just as penicillin in 1941-1944 was not the last word in the treatment of staphylococcal and other infections caused by gram-positive bacteria, so streptomycin in 1944-1946 was not the last word in the treatment of tuberculosis and of infections caused by gram-negative bacteria. The next decade was to witness the advent of a large number of other antibiotics and of synthetic compounds, to supplement the other two. Chloramphenicol and the tetracyclines were soon isolated, and came to be dubbed in certain quarters as the "broad spectrum" antibiotics, because of their activity upon the intracellular

parasites, namely the rickettsial organisms and the members of the psittacosis-lymphogranuloma group, in addition to their effect upon various gram-positive and gram-negative bacteria.

The extensive use of antibiotics, that soon came to be known in the popular mind as "miracle drugs," began to bring many problems with them. Certain severe reactions, due either to sensitization or to particular idiosyncrasies of some individuals, were to be expected. Much more general and therefore more important was the development of resistance to the older and more extensively used antibiotics, notably penicillin and streptomycin. To meet these limitations two approaches were now developed, one consisting in the combination of these with other antibiotics or with synthetic compounds, and the other comprising the isolation of new antibiotics to which the resistant organisms were sensitive. Thus came into practical use, under a variety of different names, combinations of penicillin with streptomycin, of streptomycin with dihydrostreptomycin or with PAS and INH, and a variety of others. Numerous new antibiotics were isolated. An attempt to overcome the growing resistance of the tuberculosis organism to streptomycin led to the isolation of neomycin, viomycin, cycloserine, and others. Penicillin resistance led to the isolation of erythromycin, novobiocin, oleandomycin, vancomycin, and many others.

Other groups of disease-producing microbes not sensitive or insufficiently sensitive to the first antibiotics were not overlooked. Bacitracin, a bacterial product, was found to be primarily active against gram-positive bacteria; polymyxin, also a bacterial product, was active largely against gram-negative bacteria; nystatin, an actinomycete product, and griseofulvin, a fungal product, were both active upon disease-producing fungi; a variety of other antibiotics active upon bacteria, fungi, and protozoa soon came into the field to occupy important places in human and animal therapy. The effect of antibiotics upon various plant diseases was studied and effective results obtained, as illustrated by the use of cycloheximide and streptomycin. Animal feeding and food preservation came to be benefited from the use of bacitracin, the tetracyclines and some of the older antibiotics, notably penicillin and streptomycin.

Within fifteen years after the successful introduction of the first three effective antibiotics, some 30 compounds came into practical use as important chemotherapeutic agents. A new field of science and applica-

tion was opened up. Diseases that only a generation ago were thought to be incurable became subject to successful therapy. This was true of the various diseases of childhood, as well as of pneumonia, dysentery, typhoid and typhus fevers, plague and cholera, tuberculosis, and of a variety of other infectious diseases, caused by bacteria, fungi, and protozoa.

Unfortunately, this cannot be said of diseases due to virus infections and of various organic diseases, the exact etiology of which, in many instances, still remains unknown. Although some definite progress appears to have been established in the treatment of some of these diseases, notably in cancer, the tremendous efforts made in this direction have so far not been sufficiently justified. We can hardly speak of any agent that would be active upon diseases caused by viruses, although a number of preparations that were at first believed to be effective have been isolated and described under different names. This is true, for example, of ehrlichin, xerosin, abikoviromycin, violarin, and others. A number of different antibiotics have been recorded as capable of exerting certain definite, if not always favorable, effects upon neoplasms. This is true of azaserine and DON, of sarkomycin and carzinophilin, of puromycin and mitomycin, and finally, of actinomycin.

Let me cite the last one for further illustration. As I said previously, actinomycin was the first antibiotic that was isolated in our laboratories in 1940. Although it was easily obtained in pure crystalline form and although its peculiar effects in reducing the size of the spleen were demonstrated soon after its isolation, only little attention was paid to this observation, since we were interested at that time only in its antibacterial properties. As an antimicrobial agent, it proved to be useless for therapeutic use, because it was too toxic. Several forms of it were isolated later in our own and in other laboratories throughout the world. As a matter of fact, any new screening program for antibiotic production by actinomycetes always yielded actinomycin as its first product. It is now known that a number of different species of *Streptomyces*, and as many as 10 or more per cent of all actinomycetes freshly isolated from the soil, are capable of producing this antibiotic. Various forms of it, differing in activity and toxicity, have been reported.

More than a decade passed, however, before Domagk and his group in Germany demonstrated that actinomycin has a marked effect upon certain neoplasms. According to these investigators, one form of actino-

mycin, designated as C, was able to repress the development of Hodgkin's disease. These observations attracted universal attention, including our own. We soon succeeded in isolating a new type which we designated as actinomycin D, and in demonstrating its efficacy in suppressing various experimental tumors. Much evidence soon began to accumulate, as illustrated by a symposium held March 31- April 1, 1960 at the New York Academy of Sciences. The study of the actinomycins entered a new phase. Numerous new compounds have now been isolated, either by using different organisms capable of producing them or by modifying the composition of the medium through the addition of various amino acids, such as sarcosine, proline, valine, etc. The different compounds show not only quantitative, but also qualitative differences in antitumor properties.

The field of antibiotics has now reached many ramifications. To attempt to summarize all of these would take us too far afield. It is sufficient to examine here some of them, even if only in broad outline.

Chemical Structure vs. Biological Activity

The following questions pertaining to the antimicrobial activities of antibiotics logically present themselves:

Why do some antibiotics act upon bacteria alone, others on fungi alone, and still others upon both bacteria and fungi?

Why do some affect rickettsiae and the psittacosis-lymphogranuloma group of intracellular parasites, but not the smaller viruses?

Why are the gram-positive bacteria far more sensitive to the great majority of antibiotics produced by actinomycetes and other microorganisms than are the gram-negative bacteria?

Why do some antibiotics have a marked activity upon acid-fast bacteria and not others, even those that are active upon various gram-positive organisms?

Why do some bacteria and not others develop rapid resistance to some of the antibiotics, as is the case of staphylococci *vs.* streptococci to penicillin?

Why do the rates of development of resistance differ for different antibiotics, as in the mechanism of development of resistance among sensitive bacteria to penicillin *vs.* streptomycin?

Why do some bacteria produce strains that become nutritionally dependent upon certain antibiotics, as in the case of streptomycin?

Were answers found to the above questions, one could go a long way in establishing the correlation between chemical structure and biological activity of the various antibiotics.

Even if these questions cannot be answered as yet at the present time, advantage is taken of some of the unknown properties of the antibiotics in classifying them, utilizing them, and suggesting an interpretation of their possible mode of action. The phenomenon of resistance and sensitivity of microorganisms to various antibiotics permits at least the recognition of certain close relationships among such antibiotics, if not in their chemical structure, at least in their biological activity. Thus the similarity among antibiotic preparations may be recognized long before their chemical nature has been established. Although it is now fully recognized that the mode of action of various antibiotics upon sensitive bacteria and fungi differs, too little is known about this phase of antibiotic behavior to make possible speculation upon any relationships that may exist between structure and activity.

The toxicity of antibiotics to animal tissues is known to differ greatly. This phenomenon is of great importance in any attempt to evaluate the practical potentialities of antibiotics in disease control. The actual reasons for this are largely obscure. Neomycin, for example, was shown to be an excellent agent in the treatment of tuberculosis, but so far it has not found any important place in the armamentarium of phthisiologists, largely because of its injurious nephrotoxic and ototoxic effects when administered parenterally. Different modes of administration or the supplementation of certain nutritional factors, like pantothenic acid or glucuronic acid, have been found to be the answers for the practical utilization of this antibiotic orally or topically, but so far not parenterally.

Although it has been assumed that the *in vivo* activities of the antibiotics are parallel to their *in vitro* action, there are certain instances when such parallelism is not evident. This is true, for example, of cycloserine, an antibiotic said to be more active against the tubercule bacillus *in vivo* than *in vitro*, although more recent evidence seems to suggest that this is not the case.

Attention must also be called to the fact that actinomycetes produce various growth-promoting substances, notably vitamin B_{12}, and that some antibiotics, in limited concentrations, may also exert a growth-promoting effect upon various forms of life; this property has been

taken advantage of in the nutrition of poultry, swine and other animals. These phenomena tend to complicate further our concept of the chemical structure and biological activity of antibiotics.

In order to emphasize that certain changes in the chemical structure of the antibiotic molecule may result in marked changes in its biological activity, the following illustrations will suffice:

1. When streptomycin is changed chemically to dihydrostreptomycin, whereby the carbonyl group in the central hexose unit is reduced, the characteristic antibacterial properties of the drug are retained, although there is a change in the nature of its potential toxicity. On the other hand, the treatment of streptomycin by such carbonyl reagents as hydroxylamine brings about its inactivation. The replacement of the CH_3 group in the central hexose unit (streptose) by CH_2OH, to give hydroxystreptomycin, seems to increase its toxicity without apparently interfering with its activity.

2. The modification of the aromatic aryl, the dichloroacetyl, and the CH_2OH groups in the chloramphenicol molecule results in the complete destruction of its activity.

3. The degradation of chlortetracycline to tetracycline, whereby the chlorine atom in the first ring is replaced by a hydrogen atom, results only in certain minor changes in the antimicrobial activities of the molecule, and is believed to give a less toxic compound.

4. The activity of actithiazic acid against the tuberculosis organism has been related to its thiazolidone structure.

These facts are too limited to justify any broad generalization concerning specific structure and activity of antibiotics. One point is clear, however, that the antibiotics of actinomycetes represent such a wide variety of chemical structures and of biological activities that one cannot help but conclude that we are dealing here with a new field of natural products, varying greatly in chemical composition, in antimicrobial activities, and in other biological properties that render them of great importance in human health and in human economy.

Contributions of Antibiotics to Fundamental Science

Aside from their importance in the treatment of infectious diseases in man, animals and plants, antibiotics have contributed materially to various fields of science. In the hands of qualified investigators, the antibiotics have become powerful tools for further scientific research.

This is true particularly of the fields of chemistry and biology, especially in their application to agriculture, medicine, and public health. A few illustrations will suffice:

1. The knowledge of antibiotics has contributed greatly to genetics, especially microbial genetics. The development of bacterial resistance to streptomycin and to other antibiotics proved to be a very important genetic marker for studies on the sexual recombinations both among bacteria and actinomycetes. Crossings of parental strains with rearrangement of genetic materials are usually performed with nutritionally deficient mutants of strains that normally can grow in synthetic media with a sugar as the sole carbon source. Penicillin is usually employed to concentrate and recover the induced mutants.

2. A better understanding of biological synthesis, especially of large molecules, notably proteins, nucleic acids, and cell wall material has been greatly facilitated by the introduction of antibiotics. The effect of chloramphenicol on the building of the protein molecule and the amino acid incorporation established the fact that this antibiotic uncouples the synthesis of nucleic acid from that of protein. This is also true of the effect of penicillin upon protoplast formation in *Escherichia coli.*

3. The widespread use of antibiotics has stimulated organic chemical research, and several new or very rare compounds have thus been discovered. Streptose, the first branched chain sugar to be identified in a microbiological product, was found in streptomycin; this is also true of streptidine, a base related to inositol. Only three naturally occurring polyacetylenes were known before 1950, but the study of the polyene antibiotics has increased the number to at least 25. Dichloroacetic acid and nitrobenzene, though known to the organic chemist, were found in a natural product for the first time in chloramphenicol.

4. The very fact that there is relatively little cross-resistance among the various antibiotics suggests the probability that different mechanisms of antimicrobial activity are involved. This effect of antibiotics upon microbes causing infectious diseases offers the clinician a number of possibilities in the selection of chemotherapeutic agents, alone or in combination with other antibiotics or chemical substances. The whole principle involved in the preparation of polio vaccine is based on the preservation of the polio viruses against destruction through bacterial contamination, by means of antibiotics.

5. The growth-promoting effects of antibiotics on higher animals

appear to be distinctly different from those exerted by essential growth factors or vitamins. It is not so specific as is the case for true vitamins. It involves processes heretofore scarcely recognized in nature. This stimulating effect of antibiotics has been ascribed to a disturbance of the intestinal microbial populations of the animals. A direct effect upon animal growth has also been postulated. Since antibiotics may affect adversely the bacterial population of the rumen, which assists the animal in the digestion of its cellulosic food materials, care is needed in the use of antibiotics only at a certain stage of development of the animal.

6. Human and animal semen can be preserved from bacterial attack by means of antibiotics. The same is true of the preservation of foods, especially poultry and certain vegetables. Since antibiotics do not inhibit all forms of microbial life, more than one antibiotic may, therefore, be required for proper preservation. Before the food is eaten, however, the antibiotics must be destroyed by boiling, for their constant consumption in food would tend to have certain dangerous effects upon the human body.

7. Antibiotics have introduced a new concept in our understanding of microbial life in natural environments. They have added greatly to our understanding of various aspects of biology, under natural conditions. The actinomycetes have proved to be the richest source of antibiotic producers. In 1939, on the eve of the advent of antibiotics, these organisms were considered as a rather insignificant group of microbes, largely inhabiting soils, composts, and lakes. Today, because of their ability to form antibiotics, as well as certain enzymes and vitamins, their biochemical activities and their role in nature have become problems of paramount significance.

8. Numerous other solutions of scientific problems have been made possible through the discovery of the potentialities of antibiotics. These include the extensive use of tissue cultures in biology, a better understanding of the structure of the bacterial cell, studies of superinfections, and other problems in the field of medical research.

Utilization of Antibiotics

The antibiotics have come to occupy a prominent place, together with enzymes, hormones, and vitamins, among the products of living systems that influence the growth of both higher and lower forms of life. They have become, in a way, the control mechanisms of life and

of living processes. By learning to utilize antibiotics, man has greatly simplified the problem of his survival on earth.

The potentialities of the practical utilization of antibiotics in the life of modern man are still far from exhausted. Wherever man has had to face microbes, be they injurious to his own health or to that of his herds and crops, be they destructive to his industrial products or to his foodstuffs, he has found and will continue to find in the antibiotics powerful weapons of combat.

However, as with other great discoveries that have revolutionized human life, new problems have arisen as a result of the introduction of the antibiotics. Among these are the social and economic problems that have resulted from an increase of nearly twenty years in the average life span of man. New public health problems have arisen from the practical control of many infectious diseases, notably those of childhood, of the respiratory system, of the gastrointestinal, the circulatory systems and of tuberculosis. The disturbance in the natural microbiological equilibrium by the extensive use of chemical agents that tend to eliminate certain members of the microbial population and not others, may not only stimulate the development of resistant strains but also lead to the appearance of potential undesirable mutants of microbes. The reduced natural resistance in the human and animal body as a result of the elimination of the infectious organisms also has dangerous potentialities. Some of these implications deserve particular consideration.

What Does the Future Hold?

It is believed in some quarters that the development of resistance to antibiotics suggests their reduced usefulness and gradual elimination. As new antibiotics are introduced, these, as well, are later followed by the development of resistance to them. The gloomy prophet tends to see in this the end of the antibiotic era. The optimist, however, is greatly heartened by the progress already made. He foresees in the near future the complete elimination of tuberculosis as the great enemy of man; he looks forward to the complete control of children's diseases. Such infections as undulant fever, typhoid, dysentery, cholera, plague, and venereal diseases no longer hold for him the threat that he has faced prior to the advent of the antibiotics. He even looks forward to the potential attack upon such diseases as those caused by viruses and cancer. How soon this may come about, only the future can tell.